The technique appeared to be especially well suited for newborn mice, since at later intervals, e.g., at the age of three months, details of the tissue structure were no longer as easily discerned by electron microscopy as immediately after birth. Tissue in the newborn mice is probably more loose allowing better penetration of the fixatives and embedding medium into the cartilage.

P157

PROTEOMIC DIFFERENTIAL EXPRESSION ANALYSIS OF OSTEOARTHRITIS-RELATED PROTEINS IN HUMAN ARTICULAR CHONDROCYTES: ROLE OF HSP90 IN OSTEOARTHRITIS

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Purpose: To analyze, by a proteomic approach, the protein changes that are characteristic of OA chondrocytes, and identify new OA-related proteins.
Methods: Chondrocytes were obtained from 10 OA patients undergoing joint replacement, and from 10 cartilages from autopsies without history of joint disease. Whole cell proteins were resolved by means of two-dimensional gel electrophoresis (2-DE) and stained with SYPRORuby. Protein expression patterns of gels from OA and normal chondrocyte proteins were analyzed with PDQuest 7.3.1 software. OA-related proteins were identified by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) or MALDI-TOF/TOF mass spectrometry. Western blot and immunohistochemistry analysis were performed with a polyclonal anti-Hsp90β antibody. Interleukin-1β (5 ng/ml) and Geldanamycin (1-1000 nM) were used to stimulate NO synthesis and inhibit Hsp90, respectively. Normal chondrocytes were treated with these compounds for up to 48 h prior to evaluation of NO production by the Griess method.
Results: We examined an average of 700 protein spots that were present in the different 2-DE gels. Both qualitative and quantitative changes in protein expression patterns between normal and OA chondrocytes were studied. Fifty nine protein spots were found to be statistically increased or only present in OA cells compared to the pattern of normal chondrocytes (ratio OA:N $\geq$2.0, $p<0.05$), whereas 50 were decreased in OA chondrocytes or only present in normal cells (ratio OA:N $\leq$0.5, $p<0.05$). Many of these proteins were identified, and some of them are shown in Table 1. According to their cellular role, 30% of the over-expressed proteins were involved in cellular metabolism, 11% in cell signalling, 11% in protein targeting, 9% in protein synthesis or turnover, 6% in transport processes, and 23% of them were structural proteins. From the set of under-expressed proteins, 33% of them were implicated in metabolism, 20% in cell signalling, 17% in protein targeting, 8% in transport, 5% in protein synthesis or turnover and 20% were structural. Validation of the results was performed for the NOS positive modulator Hsp90β both in cells (by Real Time - PCR and one- and two-dimensional western blotting), and in cartilage (by immunohistochemistry). Hsp90 inhibition by Geldanamycin reduces nitric oxide synthesis to basal levels (from 78.6±6.62 to 6.83±1.02 μM nitrite, $p<0.05$) in normal chondrocytes stimulated with IL-1β.

Table 1. Some of the differentially expressed proteins identified between normal and OA chondrocytes

Protein name	Ratio OA:N	Cellular role
Heat shock 90kDa protein 1 beta	4.52	Chaperone, NO synthesis
Gelsolin [precursor]	3.20	Ca-sensitive actin depolymerizer
Collagen type VI, alpha 1 chain	2.50	ECM structure, cell adhesion
Glutathione transferase omega-1	2.31	Defence and stress
78 kDa glucose-regulated protein [precursor]	2.25	Protein targeting
Glucosidase II, alpha subunit	2.12	Carbohydrate metabolism
Chloride intracellular channel protein 1	0.62	Transport

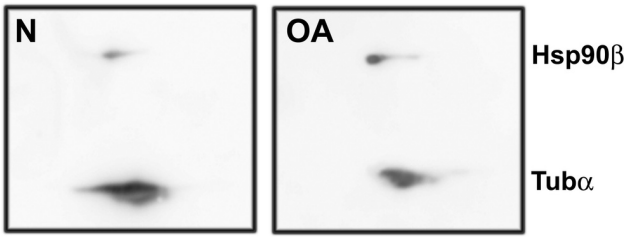

Fig 1. Two-dimensional Western blotting for examining Hsp90β over-expression in OA chondrocytes.

Conclusions: This study describes the differences between the protein profiles of normal and OA chondrocytes, pointing out the intracellular protein changes that take part in osteoarthritis. The effect of Hsp90 inhibition in preventing NO synthesis from IL-1β-treated chondrocytes suggests the role of this chaperone in the increase of NO production that occurs in OA cartilage.

P158

DIFFERENTIAL REGULATION OF UDP-GLUCOSE DEHYDROGENASE EXPRESSION AND ACTIVITY IN ARTICULAR CHONDROCYTES BY CYTOKINES AND STEROIDS

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Purpose: To investigate the mechanisms controlling gene expression and activity of uridine diphospho-glucose dehydrogenase (UGDH), a key enzyme involved in the biosynthesis of the GAGs chain precursor, UDP-glucuronic acid, by several cytokines and steroid hormones.
Methods: Articular chondrocytes (RAC) were isolated from 3-week old rabbits and incubated for 24 hours with TGF-β (3 ng/ml), IGF-I (10 ng/ml), IL-1β (1 ng/ml), IL-6 (25 ng/ml), 17β-estradiol (0.1 nM), 5α-dihydrotestosterone (1 nM) and 17β-estradiol (0.1 nM) plus 5α-dihydrotestosterone (1 nM). Total RNA and proteins were extracted and submitted to real time RT-PCR and Western blotting to determine relative expression of the UGDH gene, respectively. Spectrophotometric analysis was used to assay the activity of the enzyme. In addition, transcriptional activity of several UGDH gene promoter constructs was assayed, using transient co-transfection of RAC with wild type or mutated human estrogen receptor alpha gene (hERα66 or hERα46 respectively).
Results: We showed that steroids could exert positive regulatory effects at mRNA, protein and activity levels. In addition, we demonstrated that hERα66, but not hERα46, increased the transcriptional activity of UGDH gene. In contrast, no clear correlation between transcriptional, translational and activity of the UGDH was found for the effects of the cytokines studied. However, we observed that TGF-β enhanced the enzyme activity, whereas IL-1β, IL-6 and IGF-I were without significant effect.
Conclusions: 17β-estradiol enhanced UGDH expression in rabbit articular chondrocytes. The effect is associated with an increase of UGDH enzymatic activity. These studies provide insights into the molecular mechanisms involved in the regulation